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## REVIEW

# SIGNAL TRANSDUCTION IN THE CELLULAR SLIME MOLDS

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**Keywords:** *Dictyostelium discoideum*; cAMP; receptor; signal transduction

## I. INTRODUCTION

Intercellular communication in higher organisms depends on the central nervous system and hormones. Simple organisms such as the cellular slime molds communicate intercellularly only by using hormone-like signals. The most intensively studied species of the cellular slime molds is *Dictyostelium discoideum*. Aggregating cells of this species secrete cyclic AMP as chemoattractant, and very low concentrations of this intercellular communication signal induce molecular, behavioral and developmental changes in neighboring cells. The transduction of such a signal in the responding cell has several characteristics in common with hormone action. Binding of cyclic AMP to the cell-surface receptors of the responding cell is specific, rapid, saturable and reversible. The activated receptor regulates internal cGMP and cAMP levels and, as after hormone activation, calcium fluxes, methylation, refractory periods and down regulation are observed. Moreover, the synthesis of key enzymes might be a response to chemotactic signals.

*Dictyostelium*, as well as being simple, is also a suitable organism to grow in large quantities and, experimentally, it is a favorable subject because the amoebae are activated by chemoattractants in their single-cell phase. A large variety of mutants that may be blocked somewhere between the beginning and the end of the transduction process is available.

When the cells are still free, uptake of food takes place by phagocytizing bacteria. Also, the vegetative cells respond to simple chemoattractants, such as folic acid (Pan et al., 1972), that are secreted by the bacteria. Specificity for chemotactic signals is not so important in this stage because the amoebae feed on almost any bacterial species (Raper, 1937).

After the food supply is exhausted and there is no gradient of chemoattractant secreted by bacteria, the cells themselves start to secrete more specific chemotactic

molecules with which they attract neighboring cells. The only known chemoattractant for the initiation of the multicellular stage, cyclic AMP, attracts a few of the more advanced *Dictyostelium* species (Konijn, 1972). Chemoattractants secreted during the aggregation stage have been partially characterized in a few other species (Wurster et al., 1976, Mato et al., 1977b, Kakebeeke et al., 1978) but wait for identification.

In addition to its intercellular function, cAMP also plays a role in the enhancement of differentiation. In most species, cell differentiation is limited to two cell types: stalk cells and spores (Fig. 1).

Intercellular communication does not always result in attraction of amoebae. Also, chemorepellents are secreted by amoebae (Keating and Bonner, 1977) especially when they are still in the vegetative stage (Kakebeeke et al., 1979). The identity and function of the repellents are not known; a possible effect of repellents might be spacing of the cells to allow them larger territories to feed in. This does not explain why different species have different repellents (Kakebeeke et al., 1979).

This review will not be concerned with such topics as taxonomy (Raper, 1973; Olive, 1974), cell adhesion (Garrod and Nicol, 1981), genetics (Newell, 1978) and pattern formation (e.g. Stenhouse and Williams, 1981). We limit ourselves to detection and inactivation of chemotactic signals, the cellular response to them and the linkage between signal and response.

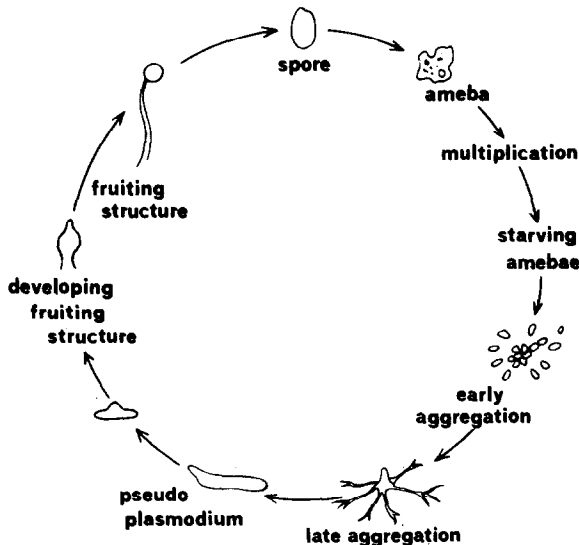


Fig. 1. Life cycle of *D. discoideum*.

## II. DETECTION AND INACTIVATION OF CHEMOTACTIC SIGNALS

Chemotactic signals are most likely detected by cell-surface receptors. cAMP does not penetrate the cell (Moens and Konijn, 1974), and lipophilic cAMP derivatives such as dibutyryl-cAMP and 8-bromo-cAMP, which may pass the cell membrane more easily than cAMP, are chemotactically far less active than cAMP (Konijn, 1974).

Inactivation of the chemoattractants serves to reduce the background concentration, and thus improves the detection of new gradients of chemotactic compounds. All chemotactic compounds are degraded enzymatically, including the repellents (Kakebeeke et al., 1979) and unidentified acrasins of *Polysphondylium violaceum* (Wurster et al., 1976), *D. lacteum* (Mato et al., 1977b) and *D. minutum* (Kakebeeke et al., 1978).

### (a) Detection of cAMP

cAMP is a chemoattractant for *D. discoideum* cells (Konijn et al., 1967) and 3 other related species, *D. rosarium*, *D. mucoroides* and *D. purpureum* (Konijn, 1972). The threshold concentration for chemotaxis in aggregative cells is 100 times lower than in vegetative cells, and aggregative cells excrete 100 times more cAMP than vegetative cells (Bonner et al., 1969). Cell aggregation in these species most likely occurs via a chemotactic reaction to cAMP.

The chemoattractant receptors are highly specific for cAMP. Any alteration of the cAMP molecule reduces the chemotactic activity. Based on the differential activity of about 50 cAMP analogs, Mato et al. (1978a) proposed a model for the cAMP—chemoreceptor interaction in *D. discoideum* cells. The high specificity is thought to be derived from 5 specific interactions between cAMP and the receptor. cAMP is fixed in the *syn* conformation by hydrogen bonds with the receptor at N<sup>6</sup>H<sub>2</sub>, N<sup>7</sup> and 3'O, and by a charge—charge interaction between the negative charge of phosphorus and a positive charge of the receptor. The adenine moiety binds additionally to the receptor by hydrophobic interactions between its  $\pi$ -electron system and a corresponding acceptor at the active site. In protein kinase type I, an intracellular cAMP receptor common in higher organisms, 5 specific interactions between cAMP and receptor are also present. However, in this case cAMP is fixed in the *anti* conformation by hydrogen bonds at 2'OH, 3'O and 5'O, by a charge—charge interaction at phosphorus and by interaction between its  $\pi$ -electron system and a corresponding acceptor at the active site (Jastorff et al., 1979).

Several research groups have found cAMP-binding proteins on the cell surface of *D. discoideum* (Malchow and Gerisch, 1974; Green and Newell, 1975; Henderson, 1975; Mato and Konijn, 1975). Binding is only detectable after cAMP has been protected against hydrolysis by cyclic nucleotide phosphodiesterase, and it can only be detected in species that react to cAMP (Mato and Konijn, 1975; Mullens and Newell, 1978). The number of binding proteins is maximal during cell aggregation. They have a high affinity for cAMP ( $K_{0.5}$ , 10–100 nM), and a lower affinity for

cAMP analogs with reduced chemotactic activity.

Green and Newell (1975), and Mullens and Newell (1978) show curvilinear Scatchard plots. This may indicate negative co-operativity or the presence of 2 types of binding site: about  $10^4$  sites per cell with high affinity ( $K_d$ , 10 nM) and roughly  $10^5$  sites with low affinity ( $K_d$ , 100 nM).

Juliani and Klein (1981), who used a photolabile radioactive cAMP analog, found only one protein which is specifically labeled under conditions that prevent hydrolysis of the analog. The molecular weight of this protein is about 45 000 daltons.

Some evidence points to a regulatory role of cAMP on the kinetic properties of the cAMP receptor. Klein and Juliani (1977) have shown that the number of binding sites that can be occupied by  $^3\text{H}$ -cAMP decreases upon pre-incubation with cAMP (down regulation). This is due to internalization of the cAMP-receptor complex or to reduction of the rate of dissociation of the complex after prolonged incubation with cAMP (Klein, 1979). Functional down regulation will be achieved only if the slowly dissociating cAMP-receptor complex is less active. This, indeed, has been indicated recently (van Haastert et al., 1981a). The activity of the receptor seems to be proportional to the frequency of receptor-cAMP interactions (rate receptor, Patton, 1961), rather than to the fraction of occupied receptors (occupation receptor). In a rate receptor a cAMP-receptor complex may produce only one response quantum. Repeated stimulation of the receptor can occur only after dissociation of cAMP from the complex. Thus, a slowly dissociating cAMP receptor is far less active than a rapidly dissociating cAMP receptor.

### *(b) Inactivation of cAMP*

Chemoattractants can only be effective if they form a sufficiently steep gradient. Local degradation is one way to steepen the gradient. A continuous attraction therefore requires a continuous degradation of the chemotactic molecules, which is achieved by enzymes excreted by the cells.

Inactivation of cAMP occurs via hydrolysis of the 3'-ribose-phosphate bond by cyclic nucleotide phosphodiesterase (PDE). Enzymatic activity is localized on the cell surface (mPDE) and in the extracellular space (ePDE) (Pannbacker and Bravard, 1972; Malchow et al., 1972). During starvation the cells excrete a protein that inhibits the ePDE (Riedel et al., 1972). The inhibitor increases the  $K_m$  of the enzyme from the micromolar range to the millimolar range (Kessin et al., 1979). The mPDE is not influenced by the inhibitor (Riedel et al., 1972). mPDE only becomes sensitive to the inhibitor after detergent solubilization (Malchow et al., 1975). Both enzymes have the same substrate specificity (Malchow et al., 1973), which indicates their relationship.

The role of mPDE may be to steepen the cAMP gradient in the immediate surroundings of the cell (Malchow et al., 1975; Nanjundiah and Malchow, 1976). The ePDE probably functions by reducing the background concentration of cAMP. The importance of PDE during cell aggregation has been shown by Darmon et al. (1978), who isolated aggregateless mutants that have a very low PDE activity. These mutants aggregate normally after addition of PDE to the medium.

*(c) Detection of pteridines*

Pan et al. (1972) report that all species examined react chemotactically towards folic acid. In the species tested by them, folic acid had a higher activity in the vegetative phase than in the aggregative phase. After testing the different moieties of folic acid (pterin, *p*-aminobenzoic acid and glutamic acid, see Fig. 2), Pan et al. (1975) suggested that the pterin moiety is the active part of the molecule. Because pteridines such as pterin, folic acid, biopterin and others are secreted by bacteria, these compounds are believed to function as a food-seeking device.

Recent observations by Kakebeeke et al. (1980a, 1980b) suggest a more complex situation. Some species also react chemotactically to low concentrations of folic acid or pterin during their aggregative phase. Furthermore, in some species, such as *D. minutum*, folic acid is more active than pterin, while in another species (*D. lacteum*) pterin is more active than folic acid. The different chemotactic reactions to folic acid and pterin indicate different receptors for these compounds.

Recently, folic-acid-binding proteins have been detected on the surface of *D. discoideum* cells (Wurster and Butz, 1980; van Driel, 1981). Post-vegetative cells contain about  $10^5$  binding sites per cell. This number decreases by less than 50% during starvation. The binding proteins are half-maximally occupied at a folic acid concentration between  $10^{-7}$  and  $10^{-6}$  M, which agrees well with the threshold concentration of folic acid for chemotaxis in this species. The binding of folic acid is not inhibited by pterin, which suggests that folic acid and pterin are detected by different receptors in *D. discoideum*.

Detection of this binding protein is possible, because the enzymatic degradation product of folic acid [2-hydroxy-2-deamino folic acid (dAFA), see section IIId] binds with about the same affinity as folic acid (Wurster and Butz, 1980). The involvement of this binding protein in a chemotactic reaction is questionable because dAFA is neither chemotactically active (Pan and Wurster, 1978) nor an antagonist of folic acid ( $10^{-4}$  M dAFA does not influence the chemotactic activity of  $10^{-6}$  M folic acid; Konijn and van Haastert, unpublished observations).

Detection of the pteridines is heterogeneous in the cellular slime molds with respect to (i) the type of receptor (folic acid type in *D. minutum* and pterin type in *D. lacteum*), (ii) the specificity of the receptor (xanthopterin is active in *D. discoideum* and inactive in *D. lacteum*), and (iii) the presence during development (folic

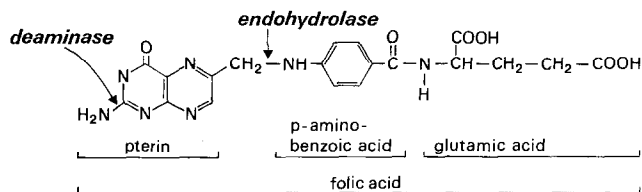


Fig. 2. Structure and enzymatic inactivation of folic acid.

acid is inactive in aggregative *D. discoideum* cells, although it is active in aggregative *D. mucoroides* cells).

#### (d) Inactivation of pteridines

The heterogeneous detection of pteridines is accompanied by heterogeneous enzymatic inactivation of these compounds. Two enzymatic reactions have been described (Fig. 2). A hydrolytic deaminase converts the amino group at the 2-position of folic acid and pterin into a hydroxyl group (Pan and Wurster, 1978). Some species contain enzymes that hydrolyze folic acid between C<sup>9</sup> and N<sup>10</sup>, yielding 6-hydroxymethylpterin and *p*-aminobenzoylglutamic acid (folic acid-C<sup>9</sup>-endohydrolase; Kakebeeke et al., 1980b).

The degradation of pteridines is further complicated by the existence of more than one deaminase which differ in chromatographic behavior and pH optima (Kakebeeke et al., 1980a). Information on the deamination of folic acid in *D. discoideum* has been increasing rapidly (Pan and Wurster, 1978; Kakebeeke et al., 1980a; Bernstein and van Driel, 1980a, b). The deaminase activity exists mainly extracellularly; minor activity is present on the cell surface and intracellularly. The  $K_{0.5}$  for folic acid is in the micromolar range. The pH optimum is broad, and peaks between 6 and 7. The extracellular deaminase activity increases during the first 3 h of starvation, whereafter it stays constant for at least 5 h. The rate of excretion of deaminase activity diminishes during starvation. Indirect evidence suggests the presence of an inhibitor of the intra- and extra-cellular deaminase activity in the membranous fraction.

The distribution of deaminase and endohydrolase in the cellular slime molds coincides with the distribution of the pterin and folic-acid types of receptor (Kakebeeke et al., 1980b). In some species the endohydrolase is the only enzyme that degrades folic acid (*D. minutum*), whereas in other species (*D. discoideum*) both deamination and endohydrolysis may occur. Still other species, such as *D. lacteum*, contain only the deaminase activity.

Three observations point to functions of pteridines other than food sensing. Firstly, slime mold cells themselves excrete pteridines (Pan et al., 1975). Secondly, in some species (e.g. *D. mucoroides*), folic acid and pterin are chemotactically active during the entire period between the onset of starvation and the beginning of cell aggregation. Furthermore, in most species, including *D. discoideum*, their chemotactic activity continues at a constant level and disappears only just before cell aggregation begins (Konijn, unpublished observation). Finally, it has been shown that pulses of folic acid accelerate development (Wurster and Schubiger, 1977; Bernstein et al., 1981).

An investigation of the excretion and activity of pteridines may shed light on the possible involvement of these compounds as intercellular communicators in the cellular slime molds.

## III. RESPONSES TO CHEMOTACTIC SIGNALS

In this section we treat the molecular and cellular responses of the cells to chemotactic signals. If not stated otherwise, data are collected from experiments on the effect of cAMP on aggregative *D. discoideum* cells. Time and concentration dependency of the reactions are analyzed and the data are summarized in Figs. 3 and 4.

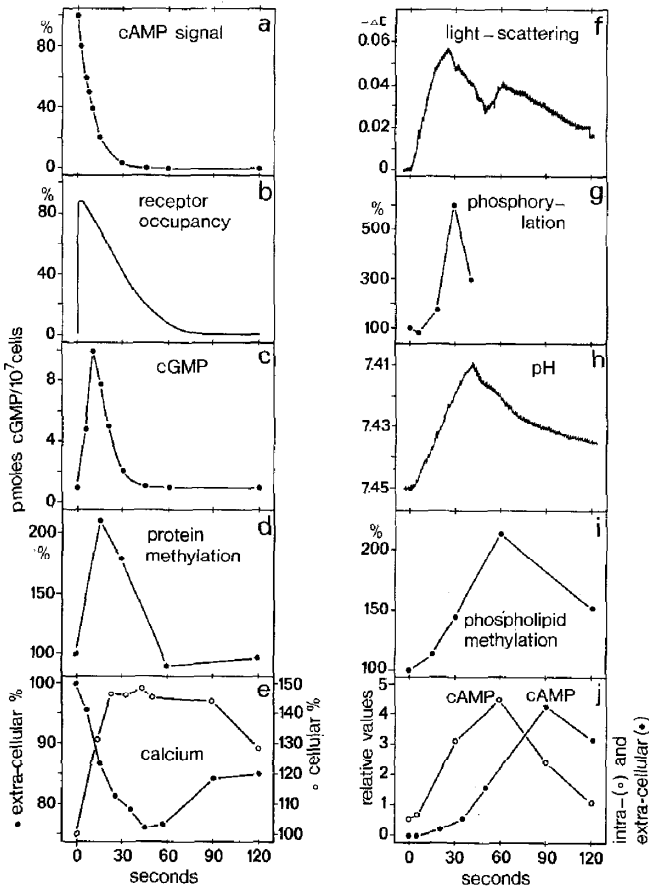


Fig. 3. The time dependency of several responses of aggregative *D. discoideum* cells to cAMP. The figures are redrawn after data taken from the literature (see text). The experiments shown in e and g were originally performed at respectively 13 and 11°C (presented time scale 0.5X, original time scale 0.4 X). a and b are unpublished measurements and calculations for  $10^{-7}$  M cAMP added to a suspension of  $10^8$  cells/ml. For phosphorylation (g), cells were stimulated with cAMP at 0 sec and lysed at the times indicated. Phosphorylation in vitro was due to an accumulation of the dephosphorylated myosin heavy chains in vivo. Other figures represent the effects of cAMP in vivo.



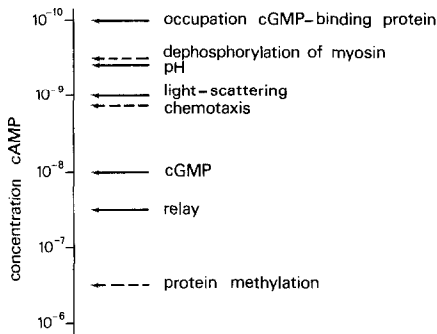


Fig. 4. Concentration dependency of several responses of aggregative *D. discoideum* cells to cAMP. The concentration of cAMP that produced a half-maximal response is indicated by an arrow. A solid arrow ( $\rightarrow$ ) indicates that half-maximal response is taken from complete dose-response curves; a dashed arrow ( $- - \rightarrow$ ) indicates an estimated value.

#### (a) Chemotaxis

Three classes of chemotactic compound have been mentioned before: attractants excreted by bacteria, attractants excreted by slime mold cells and repellents excreted by slime mold cells. These compounds have in common that they can regulate the direction in which a pseudopod is formed. When cAMP is applied with a microcapillary close to an aggregative *D. discoideum* cell, a pseudopod is induced in the direction of the capillary within 3–5 sec (Gerisch et al., 1975b). Addition of cAMP at the opposite site of the cell shortly thereafter again induces a pseudopod in the direction of the newly applied cAMP solution. These observations lead to the following conclusions. (1) Detection and analysis of a chemotactic signal by the cell and its response to the signal can occur within 5 sec. (2) Cells do not necessarily become refractory shortly after chemotactic stimulation. The threshold concentration for chemotaxis is in the nanomolar range (Mato et al., 1975). It is not yet precisely known what a cell detects. The chemoreceptor can only detect the presence or absence of a chemotactic compound. Integration of its activity over the length of a cell can give spatial information (Bonner, 1947; Mato et al., 1975), whereas information over time may provide temporal information to the cell (Gerisch et al., 1975b; cf. chemotaxis in bacteria, Macnab and Koshland, 1972).

#### (b) cGMP

One of the first biochemical changes after chemotactic stimulation of sensitive cells is an increase of the intracellular concentration of guanosine 3',5'-monophosphate (cGMP) (Mato et al., 1977a, c; Wurster et al., 1977). cGMP levels increase within 2 sec, reach a peak after 10 sec, and recover prestimulated levels after about 30 sec. All chemoattractants thus far tested induce a comparable temporal elevation of cGMP levels in sensitive cells of different species (Mato and Konijn, 1977; Wurster et al., 1978; Kakebeeke, 1980).

In aggregative *D. discoideum* cells,  $10^{-8}$  M cAMP induces half-maximal cGMP accumulation. A close correlation exists between the activity of cAMP and several of its derivatives as attractants and as inducers of a cGMP accumulation, which suggests that chemotaxis and cGMP accumulation are mediated by the same cAMP receptor (Mato et al., 1977c). cGMP levels are elevated by an activation of its synthesis by guanylate cyclase, rather than by an inhibition of its degradation by a cGMP-phosphodiesterase (Mato and Malchow, 1978). The cGMP levels are rapidly returned to prestimulated levels by a cGMP-phosphodiesterase (Dicou and Brachet, 1980) and not by excretion (Mato et al., 1977c). The enzymatic activity shows positive co-operativity; hydrolysis of cGMP is activated by cGMP which may be responsible for the spike form of the cGMP response.

cGMP may be functional by binding to and activation of intracellular proteins (Rahmsdorf and Gerisch, 1978; Mato et al., 1978b, 1979). These cGMP-binding proteins are probably not protein kinases.

#### (c) Protein methylation

cAMP induces a fast 2-fold increase of the methylation of a plasma-membrane protein with a molecular weight of about 120 000 dalton (Mato and Marin-Cao, 1979). The peak of methylation is reached at about 15 sec after stimulation (Nuske, 1980). Although dose-response curves are not shown, the lowest cAMP concentration that induces methylation is about  $10^{-8}$  M (Mato and Marin-Cao, 1979).

#### (d) Calcium

Addition of cAMP to an amebal suspension containing extracellular  $^{45}\text{Ca}$  induces a fast depletion of extracellular calcium and a concomitant increase of cell-associated calcium (Wick et al., 1978). The increase of intracellular calcium by 1–4  $\mu\text{M}$  cAMP was estimated to be about 100  $\mu\text{M}$ . Intracellular calcium reaches a maximum at about 30 sec after stimulation. The threshold cAMP concentrations that induce a response were not measured. Whether the extracellular calcium is necessary for a chemotactic response is not certain because cell aggregation is normal on agar containing 1 mM EGTA (Mato and Konijn, 1977). This observation does not argue against the involvement of intracellular calcium in the chemotactic reaction. It may well be that in the absence of extracellular calcium a cell may release calcium from intracellular deposits.

#### (e) Light scattering

A complex behavior is recorded when cells are placed in a spectrophotometer. Addition of cAMP to a suspension of aggregative *D. discoideum* cells results in a temporal reduction of light absorption (Gerisch and Hess, 1974). The light-scatter response peaks at about 30 sec after stimulation, and is evoked by low cAMP concentrations (half-maximal response by 1 nM cAMP). A light-scatter response has also been observed with folic acid in suspensions of post-vegetative *D. discoideum*

cells (Wurster and Schubiger, 1977) and with a purified attractant in *Polysphondylium violaceum* (Wurster et al., 1978). The molecular basis of light scattering is unknown.

*(f) Myosin heavy-chain phosphorylation*

Incubation of lysates of cAMP-stimulated cells with  $^{32}\text{P}$ -ATP results in an enhanced incorporation of label into a polypeptide that co-migrates with the heavy chain of myosin (Rahmsdorf et al., 1978). This has been shown to be due to a transient accumulation of dephosphorylated myosin heavy chains during chemotactic stimulation (Malchow et al., 1981). The degree of myosin polymerization increases by dephosphorylation of myosin heavy chains (Kuczmarski and Spudich, 1980). Dephosphorylated myosin peaks at about 30 sec after stimulation (the time scale in Fig. 3g is adapted to 22°C; multiplication factor  $\times 0.4$ ). Relatively low cAMP levels are sufficient to induce a response (half maximal response between  $10^{-10}$  and  $10^{-9}$  M).

Phosphorylation of myosin in vitro is inhibited by elevated calcium levels. The effect of calcium seems to be mediated by calmodulin (Malchow et al., 1981).

*(g) Changes of extracellular pH*

cAMP induces a decrease of the extracellular pH in unbuffered suspensions of aggregative *D. discoideum* cells (Malchow et al., 1978a, b). This effect is evoked at very low cAMP concentrations (half maximal response at about  $3 \times 10^{-10}$  M). The pH reaches a minimum at about 45 sec after stimulation. It is not known whether the decrease of pH is due to excretion of protons, excretion of an undissociated weak acid, or excretion of a dissociated weak base.

*(h) Phospholipid methylation*

cGMP induces a 2-fold increase of the activity of a methyl transferase in a homogenate of *D. discoideum* cells (Alemany et al., 1980). This enzyme catalyzes the transfer of a methyl group from *S*-adenosylmethionine into mono- and di-methylated phosphatidylethanolamine and phosphatidylcholine. The cGMP concentration that causes half the maximal effect is about 10  $\mu\text{M}$ , which is the maximal attainable mean intracellular cGMP concentration after stimulation with saturating cAMP concentrations.

The requirements for high stimulant concentrations are confirmed by experiments in vivo. Stimulation of aggregative *D. discoideum* cells with  $10^{-6}$  M cAMP results in a transient increase of methyl groups into phosphatidyl choline. Methylation peaks at about 1 min, and prestimulated levels are recovered after 2–3 min.

*(i) Relay*

Cell aggregation occurs via a chemotactic reaction towards compounds excreted by the cells. Cells that have entered the aggregation center excrete the chemoattractant continuously in some species (e.g. *D. minutum*). In other species, such as *D.*

*discoideum*, the chemoattractant cAMP is excreted by the aggregation center in a pulsatile manner. Cells in the surroundings of the aggregation center react to this burst of cAMP by (1) a chemotactic reaction and (2) the secretion of cAMP. Owing to this relay mechanism, steep gradients of cAMP are formed at long distances from the aggregation center.

Stimulation of a suspension of aggregative *D. discoideum* cells with cAMP results in an activation of adenylate cyclase (Roos and Gerisch, 1976). The cAMP that is produced is excreted to the extracellular space. Under conditions where cAMP is degraded fast, a delay of about 15 sec occurs between the moment of stimulation and the rise of intracellular cAMP concentrations. Intracellular cAMP achieves maximal levels at 60 sec, and extracellular levels at about 90 sec after stimulation (Gerisch and Wick, 1975). A shorter interval was observed in an extensive study where the cAMP stimulus concentration was controlled by a perfusion device (Devreotes et al., 1979a, b; Dinauer et al., 1980a, b, c).

Extracellular cAMP activates an intracellular adenylate cyclase without a delay period. The product of this enzyme, cAMP, is excreted to the extracellular space. The rate of excretion is proportional to the intracellular cAMP concentration (Dinauer et al., 1980a).

Perfusion with a constant cAMP concentration results in only a temporal activation of adenylate cyclase. The excretion of cAMP peaks after about 2–3 min and recovers to prestimulated levels after about 3–8 min. Apparently, cells adapt to a constant stimulus concentration after several minutes (see next section).

Relay occurs at a broad range of cAMP concentrations. It is detectable at  $10^{-10}$  M cAMP, and it saturates between  $10^{-6}$  and  $10^{-5}$  M; half maximal relay occurs at about  $5 \times 10^{-8}$  M (Devreotes et al., 1979b).

In an elegant experiment, Tomchik and Devreotes (1981) visualized the spatial distribution of extracellular cAMP on agar plates containing aggregating *D. discoideum* cells. They showed that the band of chemotactically responding cells is associated with a band of cAMP. Owing to the relay mechanism, the band of cAMP maintains a high concentration, even over long distances from the aggregation center.

It should be kept in mind that not all slime mold species have a relay mechanism. In species such as *D. minutum*, aggregation centers attract all cells in their territory directly.

#### (j) Refractoriness and adaptation

During the aggregation process, cAMP is periodically released by the aggregation center. Because the band of cAMP is moving outward and the cells are moving inward, the cells are first in a positive gradient profile (the cAMP concentration is increasing with time, and the highest concentration points to the aggregation center). When the maximal cAMP concentration passes the cell, the profile of the gradient reverses; the concentration decreases with time, and the spatial gradient of cAMP is no longer in the direction of the aggregation center. Because cells move

chemotactically towards the aggregation center and not backwards, they react only to the first gradient profile, and not to the second one. Therefore, it has been proposed that cells become insensitive to chemotactic stimuli after a period of chemotactic movement (Tomchik and Devreotes, 1981). This can be described as refractoriness (cells enter an insensitive stage) or as adaptation (cells react to an increase of stimulus, but accommodate to the new concentration; this accommodation causes the extinction of the response).

Paradoxically, refractoriness and/or adaptation have been found for several responses, but not for chemotaxis. Cells that are stimulated twice with  $10^{-7}$  M cAMP at a 30-sec interval react with a cGMP response only to the first stimulus (Mato et al., 1977a). However, cells react with a normal cGMP response to the second stimulus if the first stimulus is lowered to  $10^{-9}$  M cAMP. This indicates that adaptation or refractoriness of the cGMP response is fast (less than 30 sec) and dependent on the stimulus concentration.

Adaptation of the relay response has been studied extensively by Dinauer et al. (1980a, b, c). The level of adaptation depends on the magnitude and duration of the signal. That is, after prolonged stimulation with  $10^{-8}$  M cAMP, cells become insensitive to this concentration, but can still respond to higher concentrations of cAMP. Adaptation starts within 20 sec after stimulation, rises rapidly for 2–5 min, and reaches a plateau after 10 min. Cells de-adapt after removal of the signal. De-adaptation starts as soon as the signal is removed in a first-order reaction with  $t_{1/2} = 3\text{--}4$  min.

In contrast to these observations, refractoriness or adaptation has not been observed for the chemotactic reaction, for which these phenomena were proposed initially (Shaffer, 1957). Alcantara and Monk (1974) suggest that if an absolute refractory period exists it can be no longer than 12 sec. Gerisch et al. (1975b) have reported that an amoeba stimulated with 2 capillaries at a few seconds interval makes pseudopods to both capillaries. Furthermore, in the small population assay, the cells respond continuously to a chemotactic gradient (Konijn, 1970). Finally, in several species the aggregation process is a continuous movement of cells to the aggregation center (Gerisch; 1968).

#### *(k) Effects on development and differentiation*

Starvation of *D. discoideum* cells induces many biochemical changes which, after some time, lead to cell aggregation. This interphase lasts about 5–8 h, whereafter cells are maximally responsive to cAMP. Extracellular cAMP interferes with this developmental transition (Darmon et al., 1975; Klein and Darmon, 1975, 1977; Gerisch et al., 1975a, c; Yeh et al., 1978).

Pulsation of post-vegetative cells (cells starved for 1 h) with cAMP results in an acceleration of development, which can be monitored microscopically (e.g. the formation of cell clumps) or biochemically (induction of membrane-bound phosphodiesterase and the induction of cAMP receptors). In contrast to the addition of cAMP pulses, a constant cAMP stimulus results in a delay of development. Cell

clumps are formed later than in non-stimulated cells, and the cell-surface markers such as membrane-bound phosphodiesterase and cAMP receptors appear later during development. However, the extracellular phosphodiesterase activity is induced as in pulsation experiments (Yeh et al., 1978). The induction of phosphodiesterase activity by cAMP requires the stimulation of the synthesis of mRNA for phosphodiesterase (Yamasaki and Hayashi, 1979). This may suggest that extracellular and membrane-bound phosphodiesterase are under different genetical control. Some caution should be taken, however, because post-transcriptional regulation events may be involved (e.g. a constant concentration of cAMP may have a different effect than pulses on the distribution of induced phosphodiesterase activity between cell surface and extracellular space).

The potency of several cAMP derivatives to induce phosphodiesterase activity in post-vegetative cells parallels their chemotactic activity in aggregative cells (van Haastert et al., 1981a), which suggests that both processes depend on the activation of the same cAMP receptor. The shapes of the dose-response curves have been interpreted as evidence for a rate mechanism of signal transduction: the activity of the cAMP receptors is not proportional to the fraction of occupied receptors, but more likely to the frequency of cAMP-receptor interactions. Rate receptors and occupation receptors differ in several properties, of which the most important is their activity profile before equilibrium is reached (Patton, 1961; van Haastert, 1980). The activity of a rate receptor, almost immediately after administration of cAMP, is maximal and fades thereafter to a lower equilibrium response, whereas the activity of an occupation receptor is low in the beginning, and increases slowly to an equilibrium response.

Folic acid, which is mainly active in the post-vegetative phase and inactive during aggregation, stimulates development in the same way as cAMP does (Wurster et al., 1977; Bernstein et al., 1981; van Haastert et al., 1981c). This may indicate that cAMP and folic acid have parts of their signal transduction pathways to the nucleus in common. Several lines of evidence suggest that this common pathway is an elevation of intracellular cGMP levels and occupation of a cGMP-binding protein (van Haastert et al., 1981c). The signal transduction pathway, as proposed in Fig. 5, has several characteristics in common with the action of peptide hormones and steroid hormones in higher organisms. Like peptide hormones, the chemoattractants do not

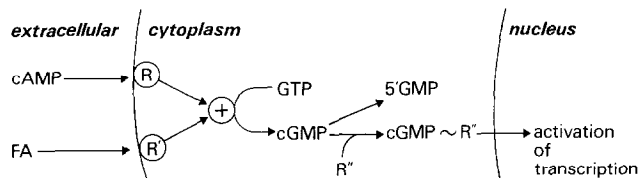


Fig. 5. Model of the signal transduction pathway for the induction of phosphodiesterase activity in *D. discoideum*. FA, folic acid; R, R', R'' are different receptors.

enter the cell, but transfer their message by activation of cell-surface receptors followed by the production of a second messenger. The action of cGMP has characteristics in common with the action of steroid hormones. The message of cGMP is protected against degradation by binding of cGMP to a soluble intracellular receptor and after transport to the nucleus this complex may affect the transcription of DNA.

#### IV. SIGNAL TRANSDUCTION FROM CELL SURFACE RECEPTORS TO INTRACELLULAR TARGETS

In the preceding section we described some processes initiated by chemoattractants. Two questions arise: does the sequence of appearance (Fig. 3) correspond to the transduction pathway to pseudopod formation and acceleration of development, and which are the targets? Acceleration of differentiation involves activation of the transcription. Pseudopod formation may require a driving force, such as actin-myosin polymerization, and a mechanism that determines the direction of the driving force.

Starting with extracellular chemoattractant, the first two steps in the transduction pathway are binding of these chemoattractants to cell-surface receptors and breaking down of the excess of chemoattractants. These receptors have high rate constants of association and dissociation (Mullens and Newell, 1978), which indicates that binding is fast. This is required because destruction of cAMP and folic acid occurs rapidly. Some arguments are in favor of 2 classes of cAMP receptor (Green and Newell, 1975; Mullens and Newell, 1978) although this has not been confirmed by others (Juliani and Klein, 1981).

Binding of the chemoattractant to the receptor may activate the receptor, which in its turn may activate some process yielding a primary response. It has been proposed that the production of primary response is not proportional to the fractional occupancy of the chemoreceptor, but may be proportional to the frequency of occupation (van Haastert et al., 1981a). The nature of the primary response is unknown. The possibility of several primary responses, originating from different receptors, cannot be excluded. As judged from the time scale of appearance, cGMP, calcium and protein methylation are candidates.

In a preliminary report, Nuske (1980) proposes that the protein methyl transferase is activated by increased intracellular calcium concentrations, and that the methyltransferase is inhibited by increased cGMP levels. The mechanism of activation of guanylate cyclase remains unknown in this sequence. Mato and Marin-Cao (1979) have found an ATP-dependent calcium pump on the outside of probably inverted vesicles derived from the plasma membrane. This enzyme, which would pump intracellular calcium to the extracellular space, is inhibited *in vitro* by *S*-adenosyl-L-methionine, the substrate of protein and phospholipid methyltransferase. It is therefore possible that chemosensory stimulation may lead to the

methylation and inhibition of the calcium pump *in vivo*. The cytoplasmic calcium levels may increase owing to a continuous leakage from the extracellular space or from intracellular deposits.

The functions of cGMP, calcium and protein methylation during chemotaxis are still largely unknown. Calcium may be involved in the contractile apparatus in analogy with that in many other organisms. Protein methylation may have a function during adaptation as has been reported for the chemotactic response in bacteria (Koshland, 1980). For cyclic GMP, two functions have been proposed: stimulation of phospholipid methylation (Alemany et al., 1980) and acceleration of development via a cGMP-binding protein (Van Haastert et al., 1981c).

Intracellular messengers for adenylate cyclase activation and myosin heavy-chain dephosphorylation have not been reported. Further, the molecular mechanisms of adaption, light scattering and pH fluctuations are largely unknown.

In Fig. 4 we have summarized the concentrations of cAMP that induce half maximal stimulation of several transduction steps. This may suggest that some processes are incompatible, such as chemotaxis and protein methylation. However, interpretation of these data is complicated by 2 phenomena. Firstly, there may be a substantial difference between the detection by a cell and the experimental observation (e.g. local fluctuations). Secondly, the non-stimulated levels and the levels that accumulate may belong to different compartments. This may explain why a half-maximal cGMP increase occurs on stimulation with  $10^{-8}$  M cAMP, whereas half-maximal occupation of a cGMP receptor occurs on stimulation with  $10^{-10}$  M cAMP (van Haastert et al., 1981b).

During the last few years several components of the chemosensory transduction pathway in the cellular slime mold have been characterized. However, important questions are still largely unanswered, such as the exact location of the chemoreceptors, possible changes in this location after interaction with the chemoattractant and the elucidation of the sequence of internal processes that promote a positive chemotactic response.

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